PERGAMON

European Journal of Cancer 39 (2003) 684–690

European Journal of Cancer

www.ejconline.com

Detection of the human papillomavirus and analysis of the *TP53* polymorphism of exon 4 at codon 72 in penile squamous cell carcinomas

O. Humbey[a], S. Cairey-Remonnay[a,b], J.S. Guérrini[a], M.P. Algros[c], C. Mougin[a], H. Bittard[d], F. Aubin[a,b,*]

[a]*Department of Cell Biology-EA 3181-Institut d'Etude et de Transfert de Gènes, University Hospital, 25030 Besançon Cedex, France*
[b]*Department of Dermatology, University Hospital, 25030 Besançon Cedex, France*
[c]*Department of Pathology, University Hospital, 25030 Besançon Cedex, France*
[d]*Department of Urology, University Hospital, 25030 Besançon Cedex, France*

Received 10 April 2002; received in revised form 14 October 2002; accepted 4 December 2002

Abstract

Human papillomaviruses (HPV) are thought to be involved in penile squamous cell carcinomas (SCC). A common polymorphism at codon 72 of exon 4 encoding either arginine (Arg) or proline (Pro) has been shown to affect HPV-mediated degradation of p53 *in vitro*, and may represent a risk factor for HPV-induced carcinogenesis. The presence of HPV DNA as well as the *TP53* polymorphism at codon 72 of exon 4 were investigated in a series of 45 penile SCC. HPV detection and typing were carried out by polymerase chain reaction (PCR) with generic primers (MY09-MY11 and FAP59-FAP64), and type-specific DNA probes. *TP53* polymorphism was further investigated using Denaturing Gradient Gel Electrophoresis (DGGE). HPV DNA was detected in 67% of penile SCC and 32% of benign lesions (BL) ($P<0.05$). Among the *TP53* amplified samples, the rate of Arg homozygosity in penile SCC was 61% compared with 68% in BL (non-significant (NS)). Our results demonstrate a strong association between penile SCC and the presence of HPV DNA. The *TP53* Arg/Arg genotype does not appear to represent a risk factor for the development of genital SCC in men, and no correlation was found between the *TP53* polymorphism at codon 72 and the presence of HPV DNA.

Keywords: Penile squamous cell carcinoma; Human papillomavirus; *TP53* polymorphism

1. Introduction

An increasing number of epidemiological studies demonstrate that human papillomaviruses (HPV) represent an important aetiological factor in a large proportion of human cancers. A particular association of HPV with the development of premalignant and malignant lesions of the uterine cervix has been established. Graham and colleagues [1] had even demonstrated that the wives of men with penile carcinomas were more prone to cervical neoplasia and *vice-versa*. It is generally accepted that oncogenic HPV types are the sexually transmitted agents common to both neoplasia. Unfortunately, very few studies have investigated penile squamous cell carcinomas (SCC) because of its rare occurence. In contrast to European and North American countries (1/100 000), South America, Africa and Asia exhibit a high incidence of penile carcinoma (10–20/100 000), which also occur in communities with high rates of cervical neoplasia [2]. The frequencies of HPV DNA in carcinomas of the penis varies from 10% [3] to 100% [4] depending on the viral detection method used and on the ethnic origin of the population analysed. Moreover, recent data have supported a role for HPV in the pathogenesis of penile SCC [5–8]. The carcinogenic effect of HPV may be explained, in part, by the transforming viral protein E6 which binds to and induces the degradation of p53 through the ubiquitin pathway [9]. It has been proposed that the existence of a common polymorphism of the tumour suppressor gene,

* Corresponding author. Tel.: +33-3-8121-8102; fax: +33-3-8121-8163.
E-mail address: francois.aubin@ufc-chu.univ-fcomte.fr (F. Aubin).

0959-8049/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0959-8049(02)00835-3

TP53, that results in either a Proline (Pro) or an Arginine (Arg) could play a critical role in the development of mucous and cutaneous SCC [10]. Indeed, the 72 Arg form of the p53 protein appears to be particularly susceptible to HPV16, HPV18 and HPV11-E6 associated degradation *in vitro*, and could facilitate the oncogenic effect of HPV infection [10]. However, these results and the importance of the *TP53* polymorphism in HPV-associated SCC are still a matter of controversy [11–13]. The aim of the present study was to evaluate the prevalence of HPV in SCC of the penis and its correlation with the *TP53* polymorphism at codon 72.

2. Materials and methods

2.1. Patients and samples

Fifty-four samples from adult patients with penile SCC, living in Eastern France, undergoing surgical resection from 1978 to 2000, were identified from our tumour registry database [14]. A subset of 45 biopsy specimens, fixed with formalin or Bouin and paraffin-embedded was finally processed for the study. Haematoxylin-eosin-stained slides were reviewed and were all confirmed as SCC. Twenty-two paraffin-embedded tissues from 22 adult patients with penile benign lesions (BL) (phimosis or Balanatis) were selected as controls.

2.2. DNA isolation

Microdissection was used to separate the tumoral cells from the normal surrounding tissues. For this study, 5–10 serial 5-μm paraffin-embedded sections were transferred to microfuge tubes. Paraffin was removed with 1 ml xylene (2×10 min) and sections were rehydrated with 1 ml ethanol 99% (2×1 min). Between each wash, tubes were centrifuged for 5 min at 1900*g* to avoid loss of material. The sections were processed for cell lysis and proteolytic digestion, overnight at 60 °C with 0.1 mg proteinase K diluted in 200 μl buffer containing 10 mM Tris–HCl pH 8.3; 50 mM KCl, 2.5 mM $MgCl_2$ and 0.45% (v/v) Tween 20. The lysate was extracted twice with phenol, and with an equal volume of chloroform–isoamyl alcohol (1:1, v/v). DNA was finally precipitated with one-tenth volume of 8 M sodium acetate and 2.5 volumes of absolute ethanol using an incubation at −20 °C for 1 h. The precipitated DNA was centrifuged, and the dried pellet was resuspended in 50 μl of water.

2.3. HPV genotyping

The DNA quality was tested by amplification of a 268-bp fragment of the *β-globin* gene using GH20/PCO4 primers [15]. Negative samples for the *β-globin* fragment were considered inadequate and excluded from the study. The presence of HPV DNA was carried out using two different sets of L1 open reading frame consensus primers. MY09/MY11 primers allowed the detection of a broad spectrum of mucosal HPV by production of a 450-bp amplicon [16]. The PCR products were then typed using the Hybridowell kit®, as we previously described in Ref. [17]. To genotype the most common HPV, the amplimers were hybridised with internal 5′ biotinylated probes for low-risk HPV6, 11, or for high-risk HPV 16, 18, 31, 33, 35, 45, 51, 52, 58 and 68. The FAP59/FAP64 primers described by Forslund and colleagues in Ref. [18] by generating amplicons of 480-bp allowed the detection a broad range of cutaneous HPV, including epidermodysplasia verruciforms (EV) associated HPV types, but also mucosal HPV types. Four specific primer sets MY5 1/2, MY5 3/4, MY8 and MY8 3/4 were also used to genotype HPV5 and 8, respectively, as described by Meyer and colleagues in Ref. [19]. DNA from CaSki cells harbouring HPV16 (ATCC CRL 1550, American Type Culture Collection, Rockville, MA, USA) was used as a positive control, while DNA from negative HPV C33A cells served as a negative control (ATCC HTB31, Rockville, MB, USA). A negative template control (water instead of DNA) was also used as a contamination control.

2.4. PCR of TP53 exon 4 and denaturing gradient gel electrophoresis (DGGE)

For DGGE analysis, *TP53* exon 4 was amplified with consensus 40nGC-clamped primers *TP53* 4.1F-R that allowed the production of 203-bp fragment [20]. The primer sequences were as follows; *TP53*.4.1F: 5′-[40GC]CCTGGTCCTCTGACTGCTCT-3′ *TP53*.4.1R: 5′-GTGTAGGAGCTGCTGGTGCA-3′. The amplification was carried out in a 100-μl volume containing 1× polymerase chain reaction (PCR) buffer (Perkin), 1.5 mM $MgCl_2$, 200 μM of each deoxynucloeside triphosphates (dNTP), 2.5 U *Taq* DNA polymerase (Ampli *Taq* Perkin-Elmer), 25 pmol of each primer and 1μg of template DNA. After DNA denaturation, 35 cycles consisting of 30 s at 94 °C, 15 s at 60 °C, 20 s at 72 °C were performed, followed by a 7-min final extension at 72 °C. The PCR products were then analysed by DGGE in the following conditions. A 16×18 cm, 1 mm thick, 8% acrylamide/Bis (37.5:1) gel with a parallel denaturing gradient range of 35–75% in 1× TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM ethylene diamine tetra acetic acid (EDTA)) was used. The gradient gel was cast using Bio-Rad's Model 475 Gradient Delivery System. Forty microlitres of PCR products were mixed with 5 μl of 2× gel loading and electrophoresed on the Dcode system. The gel was then stained with ethidium bromide in 1×TAE buffer for 5 min and visualised under ultraviolet (UV) transillumination.

2.5. Sequencing analysis

In order to confirm the results obtained by DGGE, bands were cut out from the gel and put in water in order to allow DNA elution. Then, 2 μl of product were amplified with identical primers to those used in the prior PCR under the same conditions. Finally, the amplimers were subjected to cycle sequencing with the forward primer TP53 4.1F without the 40nGC-clamped using the CEQ Dye Terminator Cycle Sequencing kit® (Beckman Coulter) following the cycling conditions: 20 sec at 96 °C, 20 s at 50 °C and 4 min at 60 °C for 30 cycles followed by a hold at 4 °C. Our test was validated on CaSki cells (Arg/Pro) and C33A cells (Arg/Arg).

2.6. TP53 *codon 72 loss Of heterozygosity (LOH)*

To test the risk of LOH in our tumour samples, we collected paraffin-embedded tissues from non-tumorous areas in our patient group. Allele-specific PCR for the codon 72 polymorphism was then carried out using two sets of primer. *TP53* codon 72 Proline sequences were detected using the primer pair *TP53* Pro+/p53− (p53 Pro+: 5′GCCAGAGGCTGCTCCCCC3′, *TP53*−: 5′CGTGCAAGTCACAGACTT3′) and p53 codon 72 Arginine using the primer pair *TP53*+/Arg−(p53+: 5′TCCCCCTTGCCGTCCCAA3′, Arg−: 5′CTGGTGC AGGGGCCACGC3′) as previously described in Ref. [10]. The results were then compared with the genotype in the tumour samples obtained from the same patient.

2.7. Statistical analysis

Pearson Chi-square test with Yates' correction when necessary was carried out using Systat* software to test of polymorphic differences according to the virological results. Statistical significance was considered at $P<0.05$.

3. Results

3.1. HPV detection

Forty-five fixed and paraffin-embedded tissues from adult patients with penile SCC were examined, and compared with 22 paraffin-embedded tissues from patients presenting BL of the penis. Mucosal and cutaneous HPV DNA were detected by PCR using the MY09/MY11 (Fig. 1) and FAP59/FAP64 primers, respectively. The overall prevalence of HPV is shown in Table 1. We were able to amplify the *β-globin* gene in 36 out of 45 SCC (80%) and in 22 of 22 controls (100%) fixed tissues submitted to PCR. Lack of *β-globin* DNA detection in the nine SCC samples probably resulted from DNA disintegration likely linked to the Bouin fixation procedure, rather than a failure of the PCR (as a positive PCR control was always obtained). Of samples found to be adequate, HPV DNA was detected in 24 of 36 penile SCC samples (67%) and in seven of 22 penile BL (32%, $P<0.05$). Mucosal HPV were found in 13 of 36 SCC (36%) and in seven of 22 penile BL (32%), while cutaneous HPV were detected in SCC in 31%. Among the mucosal HPV detected in the penile carcinomas, HPV16 was found in 9/13 samples (69%), while among the cutaneous HPV tested, HPV5 was found in 2/11 samples (18%). All mucosal HPV detected in our control samples corresponded to low-risk types HPV6 and/or 11. Four mucosal HPV and nine cutaneous HPV detected in penile SCC remained undetermined, possibly due to the limited number of HPV type probes.

3.2. Genotypic distribution of TP53 gene

TP53 genotype at codon 72 of exon 4 was investigated by DGGE after a PCR amplification using the *TP53* 4.1 primers [20]. Single bands corresponded to homozygous *TP53* codon 72 genotypes either Arg/Arg or Pro/Pro, whereas four bands were observed for the heterozygous genotype. After sequencing analysis of the single bands, we confirmed that the upper band corresponded to a

Table 1
HPV distribution in penile squamous cell carcinoma

	Total HPV (%)	HR mucosal HPV (%)	LR mucosal HPV (%)	UT mucosal HPV (%)	Cutaneous HPV (%)
SCC ($n=36$)	24 (67)	9 (25)	0	4 (11)	11 (31)
BL ($n=22$)	7 (32)	0	7 (32)	0	0

SCC, squamous cell carcinoma; BL, benign lesion; HR, high-risk; LR, low-risk; UT, untyped.

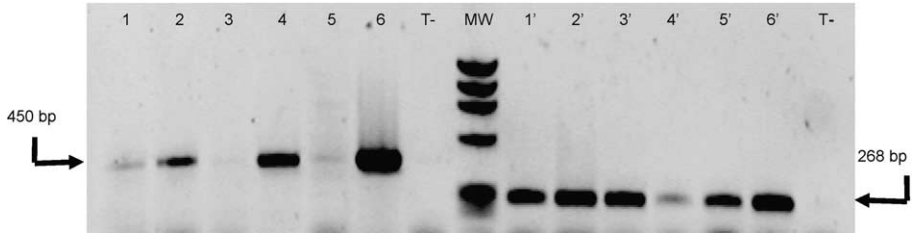

Fig. 1. Electrophoresis of polymerase chain reaction (PCR) products from different penile squamous cell carcinomas using MY09-MY11 and GH20-PCO4 consensus primers: lanes 1–5: HPV DNA samples; lane 6: HPV DNA-positive control (CaSki cells) (MW, molecular weight (ϕ174); lanes 1′-5′: *β-globin* sequence samples; lane 6′: *β-globin* DNA positive control (CaSki cells); lane T-: contamination controls (water).

Pro genotype and the lower one to an Arg genotype (data not shown). CaSki and C33A cell lines were used as Arg/Pro heterozygous and Arg/Arg homozygous controls, respectively. Representative results are shown in Fig. 2. We were able to analyse 31 SCC out of 36 that were PCR-positive for *β-globin* and 22 of 22 controls. The proportion of *TP53* codon 72 genotypes found were 61% Arg homozygous, 0% Pro homozygous and 39% Arg/Pro heterozygous in patients with penile SCC, compared with 68% Arg homozygous, 0% Pro homozygous and 32% Arg/Pro heterozygous in patients with penile BL (Table 2). Statistical analysis showed no difference between the genotypic distribution of subjects presenting penile SCC compared with controls (non significant ((NS)). In addition, we analysed the LOH in our patients: blood samples (leucocyte DNA) from 2 patients with penile SC and in normal tissue surrounding the penile SCC from 15 patients. Altogether LOH was studied in only 6 patients of the 45 patients with penile SCC because most of them were dead while the remaining tissue samples were too small or of too poor a quality (lack of β-globulin detection or insufficient peritumoral tissue). In all cases, the *TP53* genotype was similar both in penile SCC and in blood or matched normal tissue confirming the absence of LOH.

3.3. Correlation between the presence of HPV and the TP53 codon 72 Arg polymorphism

The distribution of codon 72 *TP53* genotype with respect to HPV status demonstrated a similar prevalence of Arg/Arg, Arg/Pro and Pro/Pro in HPV negative samples and in the HPV-infected patients. The statistical analysis did not indicate any significant association between HPV status and *TP53* polymorphism (NS).

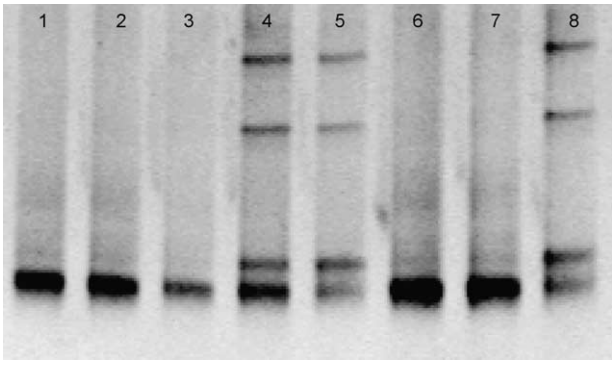

Fig. 2. Analysis of *TP53* polymorphism by parallel denaturing gradient gel electrophoresis (DGGE): lanes 1, 2, 3, 6: Arg/Arg homozygous samples; lanes 4, 5: Arg/Pro heterozygous samples; lane 7: Arg/Arg homozygous control (DNA from C33A cells); lane 8: Arg/Pro heterozygous control (DNA from CaSki cells).

Table 2
TP53 genotype distribution in penile squamous cell carcinoma

	Arg/Arg (%)	Pro/Pro (%)	Arg/Pro (%)
SCC ($n=31$)	19 (61)	0	12 (39)
BL ($n=22$)	15 (68)	0	7 (32)

SCC, squamous cell carcinoma; BL, benign lesion.

4. Discussion

Genital SCC in men are thought to be the male counterpart of cervical carcinomas and similar risk factors have been demonstrated including smoking habits, the number of sexual partners, history of condyloma, and the presence of HPV DNA [21]. Our report represents the first study investigating the presence of HPV DNA correlated to the *TP53* polymorphism status at codon 72 of exon 4 in a large series of penile SCC. A reference population of 13 samples of benign lesions of the penis (Balanatis or phimosis) was used as controls. HPV DNA detection was investigated by PCR using two sets of consensus primers, MY09/MY11 and FAP59/FAP64 allowing the detection of a broad spectrum of mucosal and cutaneous HPV. Despite the preservation problems related to the tissue source (fixed and paraffin-embedded biopsies), we could detect a high rate of viral DNA in our penile SCC population (67%) with a statistical significant difference ($P<0.05$) from those found in our control group (32%). Among these samples with HPV, mucosal HPV were predominant compared with cutaneous ones in penile SCC (54% versus 46%, NS) as in the penile BL (100% versus 0%). Among the mucosal HPV, high-risk HPV16 was the most prevalent in the genital SCC, while BL exhibited no high risk mucosal or cutaneous HPV. However, we can suppose that the HPV DNA detection in our samples is underestimated. Indeed, beside the geographical origin, the frequencies of HPV DNA in carcinoma of the penis vary depending on the sample processing and the method used for viral detection. The prevalence of HPV DNA can range from 10% [3] to 100% [4]. Levi and colleagues [6] have recently shown the difficulties encountered when evaluating the presence of HPV DNA in a series of 84 paraffin-embedded penile carcinoma. Twenty samples (18 Bouin- and two formalin-fixed tissues) out of 84 (24%) were found to be inadequate. HPV DNA was found in 28% of the remaining samples compared with 56% in 50 frozen samples tested. It is well recognised that the DNA quality from paraffin-embedded tissue is lower than DNA from fresh tissues. In our study, we could amplify *β-globin* gene sequence by PCR using the PC04/GH20 primers in 36 samples from the 45 collected (80%). In a more recent study carried out on 34 primary penile SCC, Picconi and colleagues [5] found 24 samples (71%) were positive for HPV DNA. All tissues were formaldehyde-fixed and paraffin-embedded and all DNA were amplifiable as shown by *β-globin* gene amplification.

Among the HPV detected, the high prevalence of mucosal high-risk HPV in our series agree with most reports [5,6,8,22–25]. As observed by previous authors [6,25,26], we found predominance (69%) of HPV16, but no HPV18. In contrast, previous results from Brazil [7] and from Argentina [5] indicated a high prevalence of HPV18. The differences observed between the studies could be attributed to the geographical distribution of HPV or to the lack of a representative sample. However, our study involves all penile SCC collected by the Cancer Registry of Doubs during the 1978–2000 time period in a well-defined administrative area (population 485 000) located in Eastern France [14]. The Cancer Registry of Doubs was established in 1976 and has received international recognition as demonstrated by its inclusion in the International Agency for Research on Cancer series entitled Cancer Incidence in Five Continents. In the 'département' of Doubs, cancer registration is virtually complete, and therefore technical deficiencies did not bias our results. We think that our SSC population is a representative sample of a homogenous more general population.

Among the 7 patients of our control group, 100% were infected by low-risk HPV6 and/or 11. Although there is evidence to suggest a lack of carcinogenicity of HPV6 and 11 [27], these types have previously been reported in preneoplastic and neoplastic penile lesions [4,5,7,8,28]. On the other hand, it has been pointed out that up to 10% of the penile cancer lesions have developed in association with benign lesions, that frequently contain HPV6 or 11 [2].

No previous study has investigated the presence of cutaneous HPV in penile carcinoma. We detected 31% of cutaneous HPV in penile SCC versus 0% in our control series using the FAP59–FAP64 primers as recently described by Forslund and colleagues in Ref. [18]. Such a method allows the detection of a large HPV spectrum, including epidermodysplasia verruciformis-related HPV, other HPV types (3, 10, 25, 27, 28, 29, 77, ...), which are phylogenetically grouped with the mucosal HPV types, and also low-risk (6, 11, 42) or high-risk (16, 18, 31, 52, 58, 68) mucosal HPV types [29]. Specific HPV5 and HPV8 primers [19] were used to genotype cutaneous HPV, allowing us to detect two of 11 (18%) HPV5, while no HPV8 was found. But the involvement of specific HPV types in promoting penile carcinogenesis cannot be ruled out, since amplicons generated by that PCR have not been sequenced in our study. Nevertheless, our results indicate a potential role of HPV detected by FAP59-FAP64 primers in the development of penile carcinomas.

Beside the viral type, other risk factors should be considered, like the immunological status, exposure to chemical or physical carcinogens and, genetic background. In a previous study on the same SCC population [14], we showed that over the 22-year period studied, only one male among 48 with genital SCC had been treated with high doses of psoralen plus UVA radiation. This is why we decided to study the genetic susceptibility linked to the *TP53* polymorphism, since it has been suggested that polymorphism at codon 72 of the *TP53* tumour suppressor gene might be a risk factor in the development of HPV-associated cancers [10]. Indeed, a common polymorphism of the tumour suppressor gene *TP53* that results in either a proline (Pro) or arginine (Arg) at residue 72 of exon 4 has been described [30]. The 72 Arg form of the p53 protein appears to be particularly susceptible to HPV16, HPV18, and HPV11 E6-associated degradation *in vitro* [10], and could facilitate the oncogenic effect of HPV infection. However, this finding was not confirmed by recent reports and the importance of *TP53* polymorphism in HPV-associated tumours is still a matter of controversy [11–13,31]. To our knowledge, the relationship between the *TP53* status at codon 72 and the presence of HPV in SCC of the penis has never been studied. In our study, the proportion of *TP53* codon 72 genotypes found were 61% Arg homozygous, 0% Pro homozygous, and 39% Arg/Pro heterozygous in the penile SCC, compared with 68% Arg homozygous, 0% Pro homozygous, and 32% Arg/Pro heterozygous in the penile BL. Thus, our results indicate that the *TP53* polymorphism does not seem to be associated with the development of penile SCC.

It may be argued that our results merely reflect the distribution of the *TP53* genotype of a specific geographical area, since it has been proven that *TP53* polymorphism varies according to the geographical origin, and the prevalence of *TP53* Pro allele is closely related with latitude and increases when approaching the Equator [32]. However, in two previous studies [33,34] conducted in the same geographical area, but in different groups of patients, we observed a different distribution of the *TP53* genotype using a similar DGGE analysis of exon 4 of *TP53*. Indeed, while frequencies of codon 72 *TP53* polymorphism on DNA obtained from 138 cervical smears were not significantly different between women with normal smears and women with dyskaryosis smears [33], we demonstrated that the rate of Arg homozygosity in SCC from renal transplant recipients was significantly higher than in immunocompetent patients with or without SCC [34]. The discrepancies between these studies emphasize the significance of our data and the bias of genetic variation and molecular analysis of *TP53* polymorphism could be considered as negligible. In addition, LOH may interfere with our results. Indeed, LOH is frequently observed in skin cancers on chromosome 17p, where *TP53* is located [35]. Although Storey and colleagues [10] demonstrated that LOH was not an important mechanism for overrepresentation of the *TP53* Arg genotype in cervical HPV-related cancer, our study can

not eliminate such a bias. However, we analysed LOH in 6 out of the 45 patients with penile SCC, and in all of these cases the *TP53* genotype was similar in both penile SCC and in blood or matched normal tissue, confirming the absence of LOH. Furthermore, we recently found a similar genotype after testing for *TP53* polymorphism on DNA from nine tumour specimens from renal transplant recipients compared with leucocyte DNA in the same patients [34]. The analysis of LOH was not critical in our study since we did not observe any over-representation of *TP53* Arg/Arg genotype. The analysis of LOH would have been critical if an over-representation of the *TP53* Arg/Arg genotype had been found. In addition, it has been recently demonstrated that LOH occurred more frequently in *TP53* Arg/Pro than in Arg/Arg skin tumours obtained from renal transplant recipients [36].

In summary, our results demonstrate a strong association between penile SCC and the presence of HPV DNA. However, the *TP53* Arg/Arg genotype does not appear to represent a risk factor for the development of genital SCC in men, and no correlation was found between the *TP53* polymorphism at codon 72 and the presence of HPV DNA.

Acknowledgements

O. Humbey acknowledges the supports from the Association Régionale pour l'Enseignement et la Recherche Scientifique et Technologique (ARERS), and the Ligue Nationale contre le Cancer (Comités du Doubs et de la Haute Saône).

References

1. Graham S, Priore R, Graham M, Browne R, Burnett W, West D. Genital cancer in wives of penile cancer patients. *Cancer* 1979, **44**, 1870–1874.
2. Malek RS, Goellner JR, Smith TF, Espy MJ, Cupp MR. Human papillomavirus infection and intraepithelial, in situ, and invasive carcinoma of penis. *Urology* 1993, **42**, 159–170.
3. Chan KW, Lam KY, Chan AC, Lau P, Srivastava G. Prevalence of human papillomavirus types 16 and 18 in penile carcinoma: a study of 41 cases using PCR. *J Clin Pathol* 1994, **47**, 823–826.
4. Tornesello ML, Buonaguro FM, Beth-Giraldo E, Kyalwazi SK, Giraldo G. Human papillomavirus (HPV) DNA in penile carcinomas and in two cell lines from high-incidence areas for genital cancers in Africa. *Int J Cancer* 1992, **51**, 587–592.
5. Picconi MA, Eijan AM, Distefano AL, *et al*. Human papillomavirus (HPV) DNA in penile carcinomas in Argentina: analysis of primary tumors and lymph nodes. *J Med Virol* 2000, **61**, 65–69.
6. Levi JE, Rahal P, Sarkis AS, Villa L. Human papillomavirus DNA and p53 status in penile carcinomas. *Int J Cancer* 1998, **76**, 779–783.
7. Villa LL, Lopes A. Human papillomavirus DNA sequences in penile carcinomas in Brazil. *Int J Cancer* 1986, **37**, 853–8555.
8. Gregoire L, Cubilla AL, Reuter VE, Haas GP, Lancaster WD. Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. *J Natl Cancer Inst* 1995, **87**, 1705–1709.
9. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990, **63**, 1129–1136.
10. Storey A, Thomas M, Kalita A, *et al*. Role of a p53 polymorphism in the development of human papillomavirus- associated cancer. *Nature* 1998, **393**, 229–234.
11. O'Connor DP, Kay EW, Leader M, Atkins GJ, Murphy GM, Mabruk MJ. p53 codon 72 polymorphism and human papillomavirus associated skin cancer. *J Clin Pathol* 2001, **54**, 539–542.
12. Bastiaens MT, Struyk L, Tjong-A-Hung SP, *et al*. Cutaneous squamous cell carcinoma and p53 codon 72 polymorphism: a need for screening? *Mol Carcinog* 2001, **30**, 56–61.
13. Dokianakis DN, Koumantaki E, Billiri K, Spandidos DA. P53 codon 72 polymorphism as a risk factor in the development of HPV-associated non-melanoma skin cancers in immunocompetent hosts. *Int J Mol Med* 2000, **5**, 405–409.
14. Aubin F, Puzenat E, Arveux P, Louvat P, Quencez E, Humbert P. Genital squamous cell carcinoma in men treated by photochemotherapy. A cancer registry-based study from 1978 to 1998. *Br J Dermatol* 2001, **144**, 1204–1206.
15. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature* 1986, **324**, 163–166.
16. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989, **7**, 209–214.
17. Riethmuller D, Gay C, Bertrand X, *et al*. Genital human papillomavirus infection among women recruited for routine cervical cancer screening or for colposcopy determined by Hybrid Capture II and polymerase chain reaction. *Diagn Mol Pathol* 1999, **8**, 157–164.
18. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *J Gen Virol* 1999, **80**, 2437–2443.
19. Meyer T, Arndt R, Christophers E, Stockfleth E. Frequency and spectrum of HPV types detected in cutaneous squamous-cell carcinomas depend on the HPV detection system: a comparison of four PCR assays. *Dermatology* 2000, **201**, 204–211.
20. Hayes VM, Hofstra RM, Buys CH, Hollema H, van der Zee AG. Homozygous arginine-72 in wild type p53 and risk of cervical cancer. *Lancet* 1998, **352**, 1756.
21. Dillner J, von Krogh G, Horenblas S, Meijer CJ. Etiology of squamous cell carcinoma of the penis. *Scand J Urol Nephrol Suppl* 2000, **205**, 189–193.
22. Sarkar FH, Miles BJ, Plieth DH, Crissman JD. Detection of human papillomavirus in squamous neoplasm of the penis. *J Urol* 1992, **47**, 389–392.
23. Maden C, Sherman KJ, Beckmann AM, *et al*. History of circumcision, medical conditions, and sexual activity and risk of penile cancer. *J Natl Cancer Inst* 1993, **85**, 19–24.
24. Wiener JS, Effert PJ, Humphrey PA, Yu L, Liu ET, Walther PJ. Prevalence of human papillomavirus types 16 and 18 in squamous-cell carcinoma of the penis: a retrospective analysis of primary and metastatic lesions by differential polymerase chain reaction. *Int J Cancer* 1992, **50**, 694–701.
25. Suzuki H, Sato N, Kodama T, *et al*. Detection of human papillomavirus DNA and state of p53 gene in Japanese penile cancer. *Jpn J Clin Oncol* 1994, **24**, 1–6.
26. Bezerra AL, Lopes A, Santiago GH, Ribeiro KC, Latorre MR, Villa LL. Human papillomavirus as a prognostic factor in carcinoma of the penis: analysis of 82 patients treated with amputation and bilateral lymphadenectomy. *Cancer* 2001, **91**, 2315–2321.

27. IARC. *Human Papillomaviruses. IARC edition, Vol. 64*. Lyon, IARC, 1995.
28. Barraso R, Jablonska S. Clinical, colposcopic and histologic spectrum of male human papillomavirus-associated genital lesions. *Clin Pract Gynecol* 1989, **2**, 73–102.
29. de Villiers EM. Human papillomavirus infections in skin cancers. *Biomed Pharmacother* 1998, **52**, 26–33.
30. Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J, Crawford LV. Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* 1987, **7**, 961–963.
31. Marshall SE, Bordea C, Haldar NA, *et al*. Glutathione S-transferase polymorphisms and skin cancer after renal transplantation. *Kidney Int* 2000, **58**, 2186–2193.
32. Beckman G, Birgander R, Sjalander A, *et al*. Is p53 polymorphism maintained by natural selection? *Hum Hered* 1994, **44**, 266–270.
33. Humbey, O., Aubin, F., Cairey-Remonnay, S., *et al*. TP53 polymorphism at exon 4 in caucasian women from Eastern France: lack of correlation with HPV status and grade of cervical precancerous lesions. *Eur J Obstet Gyn Reprod Biol* (in press).
34. Cairey-Remonnay S, Humbey O, Mougin C, *et al*. TP53 polymorphism of exon 4 at codon 72 in cutaneous squamous cell carcinoma and benign epithelial lesions of renal transplant recipients and immunocompetent individuals: lack of correlation with HPV status. *J Invest Dermatol* (in press).
35. Quinn AG, Sikkink S, Rees JL. Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res* 1994, **54**, 4756–4759.
36. McGregor JM, Harwood CA, Brooks L, *et al*. Relationship between p53 codon 72 polymorphism and susceptibility to sunburn and skin cancer. *J Invest Dermatol* 2002, **119**, 84–90.